STUDIES ON THE STRUCTURE OF HORSE SPLEEN APOFERRITIN

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1. Introduction

Apoferritin from horse spleen was first isolated in 1942 by Granick and Michaelis [1] who demonstrated that whereas ferritin, the iron-containing storage protein of spleen and liver, was inhomogeneous by a number of criteria including its behaviour in the ultracentrifuge, the iron-free apoprotein was homogeneous and crystallised under the same conditions and with the same crystal form as ferritin.

The molecular weight of apoferritin was found to be 465,000 by Rothen [2] in 1944 and since that time several groups have obtained values of 430,000 and 480,000 by a number of techniques [3–5]. X-ray diffraction studies [3,4] and chemical studies [6–8] have indicated that apoferritin is probably made up of twenty identical subunits of molecular weight 23,000.

In order to confirm the number of subunits present in the apoferritin molecule, and so ascertain whether they were indeed identical we have split the dissociated subunits with cyanogen bromide. There are four methionine residues per 23,000 dalton subunit [8,9] and we report here on the purification and characterisation of three peptides isolated from the CNBr cleavage reaction mixture. A preliminary report of this work has been published [10,11].

2. Materials and methods

Apoferritin was prepared as described previously [8] from horse spleen ferritin purchased from Mann Research Labs., New York, USA. Cyanogen bromide was a product of Koch Light Lab. Ltd., Colnbrook, U.K. Sephadex was obtained from Pharmacia U.K. Ltd., London, U.K. and all other materials were analytical grade where possible.

The cyanogen bromide cleavage was carried out with a 240 fold molar excess of cyanogen bromide in 90% formic acid for 24 hours. The sample was then applied to a 42 × 2.5 cm column of Sephadex, G75 and eluted at a flow rate of 36 ml/hr with 1M acetic acid using a BTL Chromapump (Baird and Tatlock,

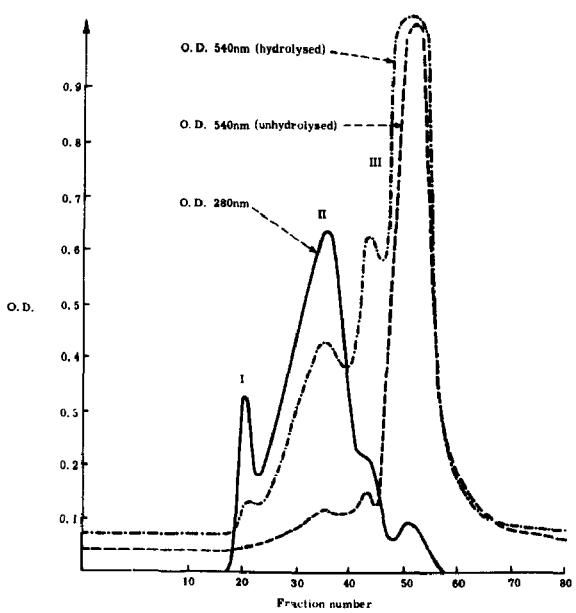

Fig. 1. Fractionation of products of cyanogen bromide cleavage of horse spleen apoferritin on Sephadex G-75. Fractions 17–22 were bulked as I, fractions 23–45 as II and fractions 48–57 as III.

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London, U.K.). The bulk of the effluent was passed through the flow cell of an LKB Uvicord II monitoring at 280 nm (LKB Producter, London) and collected in 3.6 ml fractions in a BTL Chromafrac fraction collector. 0.05 ml/min and 0.015 ml/min were continuously fed to the hydrolysed and unhydrolysed lines of a Technicon peptide analyser (Technicon Instruments, Chertsey, U.K.) and monitored after reaction with ninhydrin at 540 nm. The alignment of peaks was carried out by comparing the elution profile at 280 nm from the Uvicord with that observed when the fractions were read on a spectrophotometer manually.

Samples were lyophilised (taking care to trap the HCN produced by decomposition of the CNBr with alkali) and dissolved in small volumes of 50% formic acid for rechromatography.

Rechromatography using columns of Sephadex G75 (42 X 2.5 cm) and G50 (72 X 2.5 cm) with the same system were carried out at the same flow rate.

Amino acid analyses were performed on a Locarte amino acid analyser (Locarte Co. Ltd., Emperor's Gate, London, U.K.) on samples which had been hydrolysed overnight with 6N HCl at 110°.

3. Results and discussion

The initial fractionation of the cyanogen bromide digest on Sephadex G-75 is shown in fig. 1. Three fractions can be clearly distinguished and are marked I, II and III. The very large peaks on the hydrolysed and unhydrolysed lines of the peptide analyser for III are due to reaction of ninhydrin with cyanide formed from the breakdown of the CNBr. Rechromatography of fraction II on Sephadex G-50 gave the result shown in fig. 2. The peaks were designated I, II_1, II_2 and II_3. Components II_2 and II_3 were rechromatographed on Sephadex G-50 and component III on G-75 prior to

Table 1
Amino acid analysis of cyanogen bromide peptides

Amino acid	II_2		II_3		III		Apoferritin
	μmoles	Res.	μmoles	Res.	μmoles	Res.	Res.
Cysteic acid	0.009	0.18	0.050	0.66	0.041	0.36	3.45
Aspartic acid	0.273	5.56	0.229	3.04	0.227	2.01	21.05
Threonine	0.105	2.12	0.043	0.57	0.079	0.70	6.70
Serine	0.114	2.32	0.092	1.23	0.114	1.01	10.91
Homoserine	0.005	0.10	0.021	0.28	0.046	0.41	–
Glutamic acid	0.392	8.02	0.280	3.76	0.452	3.99	29.19
Proline	–	–	0.029	0.39	0.038	0.34	2.51
Glycine	0.198	4.04	0.149	1.99	0.227	2.01	12.01
Alanine	0.197	4.02	0.155	2.06	0.250	2.19	16.99
Valine	0.084	1.72	0.057	0.76	0.109	0.96	8.59
Isoleucine	0.066	1.34	0.025	0.34	0.070	0.62	4.25
Leucine	0.427	8.76	0.205	2.74	0.428	3.78	30.35
Tyrosine	0.954	1.10	0.063	0.42	0.026	0.23	6.07
Phenylalanine	0.092	1.88	0.097	1.29	0.117	1.04	8.92
Histidine	0.085	1.74	0.055	0.74	0.099	0.88	7.03
Lysine	0.106	2.16	0.059	0.78	0.122	1.08	10.62
Arginine	0.136	2.76	0.095	1.26	0.164	1.45	11.49

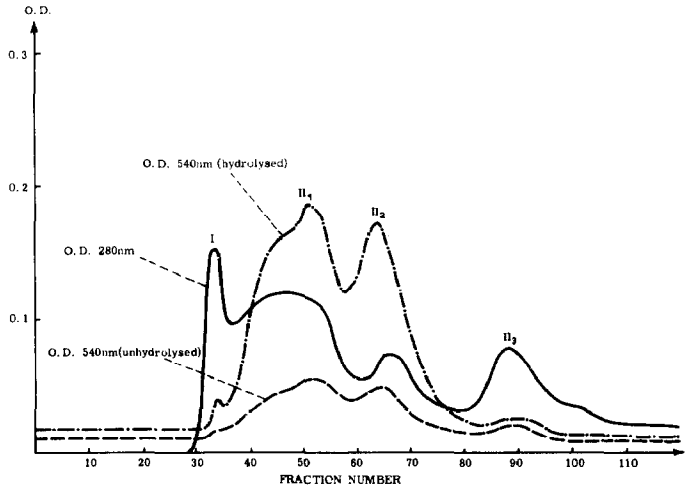

Fig. 2. Rechromatography of fraction II from initial fractionation on Sephadex G-50. Fractions 37–56 were bulked as II_1, 58–73 as II_2 and 80–95 as II_3.

analysis. They each gave symmetrical peaks.

The results of amino acid analysis of these three components are given in table 1, together with the amino acid composition of the sub-unit of apoferritin [8]. The analyses are presented as μmoles and then as residues of each amino acid, using molecular weights for each peptide calculated essentially by the method of Nyman and Lindskog [12]. The close similarity in the values obtained for II_3 and III lead us to conclude that these are probably the same peptide, II_3 being derived from some III that has not been resolved from II in the initial G-75 chromatography. Preliminary results of end group determination (R.R.Chrichton, unpublished observations) confirm this. There are also similarities between II_2 and III.

Recent results obtained in this laboratory [13] suggest that the molecular weight of the sub-unit of apoferritin may be lower than was previously thought. The complete characterisation of the remaining CNBr peptides together with the determination of the molecular weight of the sub-unit by alternative methods may enable us to establish conclusively both the homogeneity of the sub-unit and its molecular weight.

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